

*Dictyokinesis in Germ Cells, or the Distribution of the Golgi Apparatus during Cell Division.**

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[PLATES 17 AND 18.]

Introduction.

In the vast majority of animal cells so far properly studied, two categories of cytoplasmic inclusions have been identified, namely, the mitochondria and the Golgi apparatus.

The Golgi apparatus generally takes the form of an excentric juxta-nuclear system or network, composed of rodlets, platelets or beads, arranged, in many cases, around and over the surface of the centrosphere or archoplasm, in which lies embedded the centrosome. In highly differentiated cells such as the oocyte or nerve ganglion cell, the Golgi apparatus becomes dispersed into the farthestmost parts of the cell-cytoplasm, and in most cases therefore loses its relationship to the centrosome.

The equipment of an ordinary cell so far as cell organellæ are concerned, may be stated to be:—(a) chromosomes, (b) nucleoli, (c) Golgi apparatus, (d) mitochondria, (e) centrosome and centrosphere. During cell division or cytokinesis, all these bodies or organellæ are distributed between the daughter cells in some special manner. In this paper we have investigated carefully for the first time the fate during mitosis of the internal apparatus of Golgi, in a number of animals, from both Vertebrate and Invertebrate Phyla.

Material and Methods.

The material used had been prepared by one of three methods: a formalin-silver nitrate, Golgi apparatus technique; a Kopsch method, or chrome osmium and iron alum hæmatoxylin. The *Cavia* material was prepared by the Mann-Kopsch-Altmann technique (Gatenby), the *Mus testis* by Da Fano's cobalt nitrate formalin and silver nitrate modification of Cajal's method, the *Stenobothrus testis* by the latter technique (Cajal), and the Mollusc gonads by Kopsch, and by the chrome osmium methods. It will be clear therefore

* Part of the materials used for this research were purchased by a Government Grant of the Royal Society, for which we express thanks.

that we have based our results on a broad foundation so far as concerns the number of forms used, and the techniques by which the cells were prepared.

The sections were prepared by J. B. G. and studied by both of us, but R. J. L. is responsible for nearly all the drawings, and for a great share of the task of finding mitoting cells. The drawings were made with the camera lucida, and after careful discussion and an examination of numerous examples, and great care has been taken not to vitiate our results by careless observation. This has been all the more necessary because the research has offered considerable difficulties.

We have to thank Dr. Da Fano for handing over to us his material of *Mus testis* from which the case of the rat spermatocyte divisions have been described.

Note on the General Appearance of Resting and Mitoting Cells.

Everyone who has worked with silver nitrate and osmic acid techniques for the demonstration of the Golgi apparatus is well aware that these methods may occasionally prove capricious; but that such techniques really do demonstrate bodies which are not artefacts and which can be seen *intra vitam*, can easily be shown by comparing the images got by such methods, with those live cells of the gonad of *Helix* or other molluscs. This should still for ever the murmurings of the slipshod critics who have urged that silver nitrate techniques should at all times be avoided.

It is nevertheless true that quite often the formol-silver nitrate, and the Kopsch techniques fail to demonstrate the apparatus, and this applies especially to the Golgi apparatus of mitoting cells. This is the first point which we wish to emphasize: it is possible to find resting and mitoting cells side by side, with the Golgi apparatus beautifully clear in the former, and apparently altogether absent in the dividing cells; that the Golgi apparatus is *not* really absent from the latter can be shown by the examination of better impregnated material in which an apparatus will be found in mitoting cells. But it seems quite certain that during mitosis, in some cases even in the early prophases, the Golgi elements are altered in some way which makes their demonstration quite difficult.

The Golgi Apparatus in the Interkinesis Stage.

As we have already noted, our material consisted of *Stenobothrus viridulus* (a cricket) testis, of *Helix* and *Limnæa* ovo-testis prepared by Kopsch and Cajal methods, of Rat and *Cavia* testis prepared by an osmic and a formalin silver method. We possess also preparations of animals of every other class in the Animal Kingdom (excepting Protozoa) which show the

Golgi apparatus. In nearly all the cases with which we are familiar, the Golgi apparatus, when demonstrated either by an osmic or chrome osmium iron hæmatoxylin method, is found to consist of distinct rods or batonettes—the dictyosomes of Perrincito*—distributed over the surface of the centrosphere or archoplasm. (See Plate 18, fig. 14.)

In other cases the Golgi elements appear to be of a semi-liquid consistency, easily altered by anything but the most delicate treatment in both fixing and embedding materials. In many examples of mammalian cells examined by us, the Golgi apparatus seemed to be merely a cortical stainable area of the archoplasm or centrosphere, but whether this is really the case, *intra vitam*, is very difficult to decide. In other cases (Plate 18, fig. 14), separate dictyosomes or distinct cortical areas were demonstrable.

Dictyokinesis in Mus.

The division of the Golgi apparatus in the male germ cells of *Mus rattus* was worked out in preparations made by the silver impregnation method of Da Fano. The apparatus in the spermatogonia and primary spermatocytes appears as a compact mass of short black rod-like granules, embedded on a darkly-stained archoplasmic mass or centrosphere, as is shown in fig. 1, Plate 17. During the growth phase of the spermatocyte, the apparatus increases somewhat in size, and about the time when the membrane of the nucleus has broken down, preparatory to the first reduction division, the archoplasm can be seen in process of fission. At GA (in fig. 2) the archoplasm is seen separating into two portions. It will be noticed that as the two halves of this substance separate, they carry with them about an equal number of the Golgi rods or dictyosomes. In fig. 3 the archoplasm is completely separated into two constituent parts.

There is no doubt that the cause of this separation is the division of the centrosome, for the two masses move apart and come to occupy positions at opposite ends of the cell, as is shown at GA in fig. 7, which represents a late prophase of a second spermatocyte division. Between the two separated masses of archoplasm arise the fibres of the spindle, and, as the chromosomes become arranged equatorially upon this, the Golgi rods break away from the archoplasm and become scattered in the cytoplasm. Fig. 4 shows this scattering process during the metaphase. It seems almost as though some force which the archoplasmic mass or centrosome has previously exerted upon the dictyosomes is diverted during the meta- and telo-phase stages to act upon the chromosomes and draw them apart (R. J. L.). Whether or not this be the case, it can be seen that during the meta-, ana-,

* 'Arch. Ital. Biol.,' vol. 54 (1910).

and early telo-phases, the dictyosomes are scattered irregularly in the cytoplasm, and during the late telophase, when the chromosomes are completely separated off into the two daughter cells, the dictyosomes come together again and become attached once more to the archoplasm. Early and late telophases are shown in figs. 5 and 6, and the reconstructed compact form of the apparatus is shown in the case of the recently formed spermatid in fig. 8.

Both in the primary and secondary spermatocyte divisions, the process of dictyokinesis is essentially the same. About the time that the nuclear membrane breaks down, the archoplasm, with the dictyosomes, divides. During the metaphase the dictyosomes become scattered, and towards the end of the telophase they are attracted once more to the archoplasm, to the surface of which they become attached.

The rôle of the Golgi apparatus from the spermatid stage onwards has already been described by Gatenby and Woodger* in the case of *Cavia*. The greater part of the mammalian Golgi apparatus has been shown to be sloughed off during the formation of the spermatozoon, but a few dictyosomes remain attached to the middle piece. The beginning of the sloughing off process in the *Mus* is shown in fig. 9.

Dictyokinesis in Cavia.

Dictyokinesis in *Cavia cobaya* is, with few exceptions, essentially the same as in the case in *Mus*. The process in this mammal has been studied in preparations made by Weigl's modification of the osmium tetroxide method of Kopsch.

During the early phases in the development of the spermatocyte, the archoplasm to which the dictyosomes are attached shows a remarkable frothing. This is shown at "b" in fig. 16, and again at VV in fig. 12. Small "bubbles" appear to arise peripherally upon the archoplasm; they increase in size and, owing to the fact that later they disappear, it would seem that they burst and discharge their contents into the cytoplasm. Peripherally, these bubbles are surrounded by a substance which stains black with osmium tetroxide. At the end of the diakinetik stage of the chromosomes the formation of these "bubbles" upon the archoplasm ceases, and the dictyosomes are then seen as semilunar rods attached peripherally to the archoplasm, as is shown in fig. 16 at "a."

Before the nuclear membrane disappears the archoplasmic mass, which has enlarged during the growth period, begins to divide in exactly the same manner as in *Mus*. The beginning of division is shown in figs. 13 and 14.

* 'Quart. Jour. Micr. Science,' 1921.

The divided apparatus is shown in fig. 15. In fig. 17 is seen a metaphase stage, the Golgi elements scattered in the cytoplasm appear as annular bodies composed of two semi-lunar rods. Mitochondria have been drawn in this figure as they appear in preparations counter-stained with Altmann's acid fuchsin. During the telophase, when the chromosomes are completely drawn apart, the dictyosomes are attracted once more to the astrosphere. Their movement towards the astrosphere at this stage is shown in fig. 18.

The second spermatocyte division takes place in exactly the same manner as the first. The divisions of the archoplasm, and its movement to the opposite ends of the cell during the prophase, appears to take place with considerable rapidity, as very few cells are found in these stages, and it will be seen from the figures that but little change occurs in the chromosomes during fission and separation of the archoplasm.

Division of the Chromatoid Body in the Mammals Mus and Cavia.

At the same time as the Golgi apparatus of the mammalian spermatocyte is in process of division, the chromatoid body breaks up into two equal parts. This process is best seen in the Weigl-Kopsch preparations of Cavia, in which, after staining with acid fuchsin, the chromatoid body appears coloured similarly to the mitochondria. In fig. 13, which shows the beginning of dictyokinesis in the primary spermatocyte of Cavia, a single large chromatoid body (CB) is seen. In fig. 14, where the archoplasm appears very nearly divided into two parts, the chromatoid body has divided, and the two parts are just separating. They are further apart in fig. 15, while, in the metaphase stage shown in fig. 17, they occupy positions on opposite sides of the chromosome group. A similar stage in the spermatocyte of Mus is shown in fig. 4. At the telophase, the two chromatoid bodies become separated off into the newly forming cell, as is happening in fig. 18. Consequent upon the repetition of this process in the second spermatocyte division, each spermatid has a single chromatoid body directly derived from that of the spermatogonium.

Dictyokinesis in Molluscs.

The fundamental nature of the dictyokinesis in the germ cells is shown by the similarity of the process in animals so widely apart in their systemic position as the Mollusca and Mammalia.

At fig. 19 is drawn a primary spermatocyte of Helix as it appears in Flemming-without-acetic preparations, stained by iron alum hæmatoxylin. The chromosomes have reached their diakinetik stage, and mitochondria, which previously were rounded, have metamorphosed to form rodlets.

The archoplasm is seen just dividing, and carrying with it the semi-lunar Golgi rods. The two parts of the apparatus are carried to opposite ends of the cell by the centrosomes, and the spindle arises between them in the normal manner.

An early anaphase in the primary spermatocyte of the mollusc *Limnæa* is shown in fig. 20, which is drawn from a Kopsch preparation. It will be noticed that, while the chromosomes are being drawn apart, the dictyosomes are breaking away from the asters. Later they become more scattered, and then, when the chromosomes have reached the poles of the spindle, the dictyosomes are drawn once again to the archoplasm.

The same process is repeated during the second spermatocyte division, so that, in the spermatid, the Golgi apparatus is in the clumped condition. In the formation of the spermatozoon, it is sloughed off along the tail, and is seemingly absent altogether in the mature spermatozoon.

Dictyokinesis in Stenobothrus.

Dictyokinesis, as observed in the spermatocyte of the cricket, *Stenobothrus viridulus*, differs from that described for the previous animals, in that the Golgi elements break away from the archoplasm at a very early stage in the development of the spermatocyte. In Cajal preparations, the apparatus in the spermatocyte appears first as a number of black granules around the archoplasm. Soon these granules break away and scatter in the cytoplasm, leaving the archoplasm, as is shown in fig. 10.

The end of a typical first spermatocyte division is shown at fig. 11. It will be seen that the Golgi elements are fairly evenly distributed in the secondary spermatocyte. After the second spermatocyte division, many of the dictyosomes become once more attached to the archoplasm, and they remain thus in the spermatid.

Discussion.

It seems to be agreed by most observers that, during mitosis, the chromosomes divide longitudinally, that is, if they are elongated in shape. Bolles Lee,* in a recent paper, has dropped a bombshell into the camp of the chromosome theorists and Mendelians, by suggesting that "there is no longitudinal splitting. The division is a transverse one, brought about by a folding of the chromosomes at their middle, and their ultimate segmentation at the bend there formed." It is not our intention at the present juncture to join in this discussion, but, for the purposes of this paper, it may be said that, whether the division of the chromosomes be transverse or longitudinal, it is at least a very equal one quantitatively.

* 'Quart. Jour. Micr. Science,' 1921.

In this present paper, we have shown that, in animal germ cells, the Golgi apparatus may during interkinesis be spread throughout the cell cytoplasm, as in *Stenobothrus*, or it may be excentric and juxta-nuclear, as with most of the forms herein described. In a previous communication, one of us showed that, in the case of *Limax agrestis*, the number of dictyosomes or Golgi (nebenkern) rods in the spermatocyte is almost always eight, and, after the two maturation divisions, each of the four spermatids has two rods.

The size of the rod in the spermatid is the same as that in the spermatocyte, and this, with the evidence brought forward in this paper, shows that each rod passes through the maturation divisions undivided. It had previously been shown that the number and size of the dictyosomes or Golgi rods in such a mollusc as *Helix aspersa* may vary greatly.*

Multiplication of the number of the Golgi rods may take place during the interkinesis stages, as was demonstrated clearly in the case of the *Limnæa* egg,† but no actual division of individual rods seems to occur at cytokinesis, but only a sorting out of whole rods. This is well shown in Plate 18, figs. 19 and 20 of this paper, and was first worked out by J. A. Murray.‡

When we come to the case of some of the mammals, it will be noted that, even though the molluscan dictyokinesis may be haphazard and unequal, that of the mammal is even more irregular. That there is a true dictyokinesis or distribution of Golgi elements during mammalian cytokinesis cannot be doubted, but there are several relative points which should be brought to notice. In the first place, the Golgi apparatus of such forms as *Mus* and *Cavia* often appears to be a mere cortical layer plastered upon the surface of the centrosphere, separate dictyosomes or rods of any kind being apparently absent. In other cases there are distinct dictyosomes, but these are rarely so well marked as in molluscan germ cells. Dictyokinesis in the mammalian germ cells studied by us is never so much a sorting out of distinct rods as in molluscs, as an irregular breaking up of possibly only that part of the archoplasm or centrosphere in close proximity to the more cortically disposed Golgi substance. It is only right to point out, however, that we have found some very clear metaphases where the Golgi elements were composed of discrete dictyosomes, as in Plate 77, fig. 3.

But we come to another point; perusal of such work as that of Lenhossék§ on the rat will show that such observers figure maturation metaphases with a single free unbroken centrosphere at one pole of the

* See 'Quart. Jour. Micr. Science,' vol. 62 (1917), Plate 32, fig. 25, and Plate 33, fig. 32.

† 'Quart. Jour. Micr. Science,' 1919.

‡ 'Zool. Jahrb.,' vol. 11.

§ Quoted and figured by Doncaster in his 'Cytology.'

spindle; in other words, according to Lenhossék and such workers, the centrosphere does *not* divide as we have drawn in Plate 17, figs. 1-7, of the present paper. But in our own preparations of the rat, we never definitely found such an undivided centrosphere at metaphase, and can only conclude that Lenhossék is wrong. Our figures 1-7 of the rat are carefully drawn and are typical of many examples we have studied.

In molluscs the Golgi rod or dictyosome is evidently a compact and definitely shaped body possibly formed of some proteid substratum, but in the case of the mammalian germ cell the Golgi element appears to be more fluent, and less able to keep its shape during dictyokinesis. That this is so, seems all the more likely from evidence procured by examining the results of different fixing techniques on the apparatus. The Golgi apparatus dictyosome of the mammalian germ cell during the metaphase always appears to show a tendency to become spherical or ovoid, which indicates that it must be nearly of the same viscosity as the ground cytoplasm in which it lies, assuming of course that the protoplasm is still a sol at this period.

Chambers' work on the dividing sea-urchin's egg may not apply to maturing germ cells, but we do not know at present. At all events it seems indicated that the Golgi substance of the mammalian germ cell is of a more liquid nature than that of the mollusc.

There is then another matter to which we wish to turn: although many of the older workers figured Golgi rods or dictyosomes in the resting cell, they rarely found any apparatus during kinesis. Take for instance the work of Tullio Terni.*

It is quite certain that some change comes over the Golgi elements during cell division; in molluscs, insects and mammals, in certain worms such as *Saccocirrus*, and in all the examples we have observed, it has been found that it is possible to find mitoting and resting cells side by side, in *either* of which the apparatus may apparently be absent—but only apparently.

The case of the mollusc spermatocyte is classical; short mordanting in chrome osmium, and curtailed staining in iron hæmatoxylin is always sufficient to show the Golgi rods of resting cells, but this treatment will often prove insufficient to bring into evidence the apparatus of mitoting cells. But prolong the mordanting, and the staining, and the story becomes quite different—this is what observers like Terni have failed to do.

To explain this behaviour of the Golgi element is difficult, yet in it we see possibly the one solution. In the case of the chrome osmium technique it seems that the difference is due to a withdrawal from the Golgi elements at the prophase and metaphase, of some lipid substance to which the heavier

* 'Arch. f. Zellf.,' vol. 12 (1914).

and more facile staining of the resting stage is due. In the case of the Weigert stain of medullated nerve fibres, the hæmatoxylin forms a lake with oxidized ($K_2Cr_2O_7 + OsO_4$) lipoids in the sheath. We think that such may explain the behaviour of the Golgi substance at cytokinesis. The lipoids thus withdrawn from the Golgi elements might serve some purpose during cell division. In this connection one remembers Boveri's view that the archoplasm or centrosphere supplied the material for the formation of the mitotic spindle.

The Significance of Dictyokinesis.

It has been shown here that the process of dictyokinesis is generally extremely haphazard, and in contrast with karyokinesis does not entail any sort of fission of individual elements as occurs with the chromosomes, but merely an unprecise sorting out of parts of the Golgi reticulum, between the two daughter cells.

It seems indicated that the Golgi apparatus is an integral and necessary part of the cell constitution, but during cytokinesis or cell-division, there is to be found in connection with the Golgi apparatus nothing comparable with the exact division of the chromosomes. The distribution of the Golgi apparatus between the daughter cells, as we have shown, takes place by a more or less rough process of sorting out of individual pieces or spheres formed by disintegration of the apparatus during the prophase of mitosis, and this process entails no division of the individual elements or dictyosomes, as occurs in the chromosomes of a somatic mitosis.

All this seems to indicate that the Golgi apparatus takes a part less precise and less important, in the hereditary processes of the cell, than that fulfilled by the chromosomes.

DESCRIPTION OF PLATES.

Explanation of Lettering.

AR ... Archoplasm.	M ... Mitochondria.
CB ... Chromatoid body.	VV ... Vacuoles associated with the
CH ... Chromosomes.	Golgi apparatus and Archo-
GA ... Golgi apparatus.	plasm.

PLATE 1.

Figs. 1 to 9 were drawn from preparations of the testis of the rat which had been fixed by Da Fano's method and counterstained with carmine.

Fig. 1.—Spermatogonium, showing the Golgi apparatus at GA in the compact form.

Fig. 2.—Late prophase of the primary spermatocyte division. The nuclear membrane has broken down, and the Golgi apparatus (GA) is dividing.

Fig. 3.—Primary spermatocyte division, showing the two parts of the Golgi apparatus (GA) moving apart.

Fig. 4.—Metaphase in primary spermatocyte division. The chromosomes (CH) are arranged equatorially and the Golgi rods are scattered in the cytoplasm. The chromatoid body is shown at CB.

Fig. 5.—Early telophase of primary spermatocyte. The Golgi elements are scattered irregularly.

Fig. 6.—Late telophase of second spermatocyte division, showing the two separated groups of chromosomes (CH), and the dispersed dictyosomes (GA).

Fig. 7.—Late prophase of secondary spermatocyte division, showing the two archoplasmic masses with adherent Golgi apparatus (GA) at opposite poles of the cell.

Fig. 8.—Spermatid with reconstructed compact form of the Golgi apparatus (GA).

Fig. 9.—Spermatozoon with the Golgi apparatus before becoming sloughed off.

Figs. 10 and 11 were drawn from Cajal preparations of the testis of *Stenobothrus viridulus* stained with fuchsin.

Fig. 10.—Spermatocyte, showing the archoplasm before division, and the numerous scattered Golgi elements (GA).

Fig. 11.—Late telophase of first spermatocyte division, showing the elements of the Golgi apparatus (GA) fairly equally distributed in the secondary spermatocytes.

PLATE 2.

Figs. 12 to 18 were drawn from Weigl-Kopsch-Altmann preparations of the testis of *Cavia*. Mitochondria are shown only in fig. 17, but are present at all stages.

Fig. 12.—Spermatocyte showing the curious vacuoles (VV) associated with the Golgi apparatus.

Fig. 13.—Primary spermatocyte at late prophase with the Golgi apparatus beginning to divide and a single chromatoid body (CB).

Fig. 14.—Later stage of the prophase, the Golgi apparatus is in process of fission, and the chromatoid body (CB) has just divided.

Fig. 15.—Primary spermatocyte with the apparatus (GA) divided into two parts. The nuclear membrane has just broken down, and there are two chromatoid bodies present.

Fig. 16.—Two forms of the Golgi apparatus, as found in the spermatocytes of *Cavia*. In (a) it consists of a number of semi-lunar dictyosomes attached peripherally to the archoplasm. In (b) it is much more darkly impregnated with the osmium tetroxide and a number of frothings appear on its surface.

Fig. 17.—Metaphase of first spermatocyte division. The Golgi elements (GA) are scattered unevenly in the cytoplasm, the chromatoid bodies are seen at CB, and granular mitochondria (M) are fairly evenly distributed throughout the cytoplasm.

Fig. 18.—Telophase of second spermatocyte division, showing the Golgi elements (GA) moving towards the archoplasm, and the chromatoid bodies at CB.

Figs. 19 and 20 show the spermatocytes of Molluscs. The former is from a Flemming-without-acetic preparation, stained by iron alum hæmatoxylin: and the latter from a preparation by the Kopsch method.

Fig. 19.—Primary spermatocyte of *Helix aspersa* at the diakinetik stage. The Golgi apparatus has just divided, and the two archoplasmic spheres are beginning to separate. Filamentous mitochondria (M) are seen scattered throughout the cytoplasm.

Fig. 20.—Metaphase of the first spermatocyte division of *Limnaea stagnalis*, showing the Golgi elements (GA) attached peripherally upon the asters at both poles of the nuclear spindle.